



# The influence of gentle interactions on avoidance distance towards humans, weight gain and physiological parameters in group-housed dairy calves



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## ABSTRACT

The quality of the relationship between cattle and their caretakers is important for animal welfare and productivity. Nevertheless, the influence of gentle interactions on group-housed dairy calves has not been thoroughly studied so far. We examined the effects of 42 min of gentle interactions (stroking, gentle talking) during the first 14 days of life on female Holstein-Friesian calves. The control calves experienced only routine management. We measured the calves' avoidance distance towards a familiar person after the treatment phase ( $17.7 \pm 2.4$  days old, groups of 12–14 animals), after disbudding ( $32.3 \pm 4.4$  days old, groups of 26 animals) and after weaning ( $86.2 \pm 5.1$  days old, same group composition as after disbudding). In addition, we measured heart rate variability (HRV) during resting after the treatment and after weaning and concentrations of faecal cortisol metabolites (FCM) after weaning, and we calculated the average daily gain in body weight from birth until after weaning.

Calves frequently showed neck stretching (occurrence in 37% of all occasions) and play behaviour (9%) during the treatment. After the treatment phase, avoidance distances were lower in stroked calves than in controls ( $p = 0.02$ ). After disbudding, avoidance distances were higher than before in both groups ( $p < 0.001$ ), and there was no longer a significant difference between the groups (interaction of treatment and testing day,  $p = 0.002$ ). After weaning, avoidance distances tended to be lower in stroked calves than in controls ( $p = 0.07$ ). Average daily gain was higher in stroked calves than in controls ( $p = 0.05$ ; up to 7%, depending on milk provision; interaction of treatment and milk provision,  $p = 0.05$ ). There was no significant difference between groups with regard to heart rate variability or faecal cortisol metabolites.

The treatment was effective in reducing the calves' fear of humans in the short term and partly in the medium term. The higher average daily gain in stroked calves is an important economic aspect, as heifers with a high average daily gain have been shown to have a higher milk yield later in life.

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**Abbreviations:** AD, avoidance distance; ADG, average daily gain (in body weight); HRV, heart rate variability; SDNN, standard deviation of the NN interval; RMSSD, square root of the mean squared differences of successive NN intervals; HFnorm, normalized power for high frequency; LFnorm, normalized power for low frequency.

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## 1. Introduction

A good relationship between cattle and humans is important for ethical reasons (Balcombe, 2009; Green and Mellor, 2011), as it leads to reduced stress responses to routine management practices (Lensink et al., 2001; Waiblinger et al., 2004), and thus, improved welfare. It may further have economic benefits, such as higher productivity, and increase work safety (reviewed in Waiblinger et al., 2006). In addition, consumer demand for welfare-friendly products rises (Bayvel et al., 2012; Buller, 2013), offering additional economic benefits via label production.

Gentle tactile interactions are a means of improving the relationship between animals and humans (Nowak and Boivin, 2015) and have the potential to elicit physiological reactions that can be summed up as 'anti-stress effects', for instance a decrease in heart rate (Schmied et al., 2008b), which may be linked to the release of oxytocin (Uvnäs-Moberg, 1998). Potential positive effects of gentle interactions during the first weeks of life on the relationship between group-housed dairy calves and humans and on the basic physiological state of the calves have not been thoroughly studied so far, despite the general ban of single housing of calves over eight weeks of age by European legislation (council directive 97/2/EC). Although Sato et al. (1984) investigated the effects of stroking on reactions to humans and social behaviour of group-housed calves, the treatment was conducted while the calves were still housed singly, and it was very intense (daily 90 min during the first 35 days of life). Boissy and Bouissou (1988) studied the influence of long-term handling, including brushing, on fear of humans and ease of handling of group-housed calves and heifers in different age groups. Lensink et al. (2001) and Raussi et al. (2003) investigated the effect of gentle interactions on fear of humans and reactions to handling and short transport in individually and pair-housed but not on group-housed calves. Schütz et al. (2012) and Stewart et al. (2013) studied the influence of positive and mildly negative interactions with humans on the relationship to humans and reactions to painful routine husbandry procedures calves housed in groups of five animals. However, they did not include a non-handled control in their tests for short-term effects; differences at that point in time may therefore result exclusively from the negative treatment.

A further important aspect that merits consideration is that weight gain may be influenced by the type of handling experienced and thus by the level of fear of humans (e.g. in veal calves: Lensink et al., 2000a). There might also be direct physiological effects of stroking on weight gain (Holst et al., 2005). Up to now, no study has investigated the influence of gentle interactions on weight gain in group-housed dairy calves.

In a previous experiment conducted on a research farm, we investigated the effect of gentle interactions on calves of a dual-purpose breed housed in groups of four in comparison with a non-handled control (Lürzel et al., in press). There were only a minor number of significant differences between the groups in parameters indicative of the quality of the relationship to humans and of stress. Both control and stroked calves showed very little fear of humans, which points towards a ceiling effect.

The aim of our present study was to test the effects of gentle interactions during the first two weeks of life under conditions found on large commercial dairy farms. We worked with Holstein-Friesian calves kept in larger groups and fed via an automatic milk feeder soon after birth, a condition not yet investigated so far. We further reduced the number of tests and sampling procedures in order to minimize the contact between the calves and the experimenters, apart from interactions during the treatment. We hypothesized that calves that experienced additional gentle interactions during early life would show less avoidance behaviour towards a human in the short and medium term. Furthermore, we expected lower concentrations of faecal cortisol metabolites and a higher heart rate variability (HRV) in stroked calves compared to controls as well as a higher average daily gain in body weight in stroked calves than controls.

## 2. Methods

### 2.1. Animals, housing and management

The experiment was carried out with 104 Holstein-Friesian calves (91 females, 13 males) at a commercial dairy farm in

eastern Germany. The farm kept a herd of about 1200 Holstein-Friesian cows. Calves were born between June and August 2013. After birth, they were separated from the dam within one hour (except for night calvings), and each calf was weighed and its umbilical stump disinfected. Each calf was tube-fed 3 l of colostrum and housed in a straw-littered single pen in the calf barn (Table 1) that allowed for visual and limited tactile contact to one other calf. The calves stayed in the single pens for 3–10 days and subsequently were moved through a series of straw-littered group pens (Table 1). In the different pens, the calves were fed different amounts of milk or milk replacer (Table 1). Because the groups from pens 4 and 5 were combined to one group in pen 6, the milk ration of every second group of 12–14 calves was reduced one week of age earlier than the other groups' milk ration. Hay, concentrate and water were available *ad libitum* in the group pens as well as a salt block. In pen 6, calves were disbudded at an age of  $31.3 \pm 4.3$  days (mean  $\pm$  SD) without anaesthesia or analgesia with a heated disbudding iron while being restrained in a head-lock device, according to the usual farm practice.

The age range within a group of 26 animals was between 6 and 15 days. Although the groups were largely stable, nine animals left the group to which they were originally assigned in the course of the study: Five calves were sold before the end of the experiment, two calves died, and two were moved to the next younger group because of unsatisfactory growth. Calves that had switched groups were excluded from the following tests.

Calves were kept under the farm's usual conditions, including visual contact to humans during bucket feeding and cleaning of pens and tactile contact mostly in the context of feeding but also of rehousing and veterinary treatments. There were three stockpeople (two females, one male) responsible for calf management; approximately five further stockpeople provided routine post-partum care at night. The stockpeople that cared for the calves continued to do so during the experiment without changing their routines; they as well as the other stockpeople were however instructed not to provide any additional contact like letting the calves suck their fingers or stroking or talking to them. An exception to that rule was the teaching phase, during which presentation of fingers for sucking helps to increase the calves' sucking motivation and thus, facilitates the learning process in order for the calves to independently access the automatic milk feeder. The disbudding procedure was conducted by two stockmen that had no other duties concerning the calves.

All experimental procedures applied during the course of this study were discussed and approved by the institutional ethics committee in accordance with guidelines for Good Scientific Practice and with national legislation.

### 2.2. Experimental design

Each group of 26 calves was assigned to one of the two experimental conditions in the following order: control group (C1), gentle contact group (G1), gentle contact group (G2), control group (C2). All groups were managed according to usual farm routine. In addition, the gentle contact groups experienced 3 min of gentle interactions with one of the two experimenters per day (with each animal stroked equally often by each of the experimenters) during the first 14 days of life (see Section 2.3).

Testing (Fig. 1) took place after the end of the treatment period ( $17.7 \pm 2.4$  days of age; pen 4), one day after disbudding ( $32.3 \pm 4.4$  days, pen 6) and after weaning ( $86.2 \pm 5.1$  days, young stock barn). After the treatment period (groups of 12–14 animals), an experimenter measured the calves' avoidance distances (ADs) in the morning (see Section 2.5). It was not possible to take a baseline measure before the treatment because it started on the first day of life, when AD tests are not yet feasible. In the afternoon of the same

**Table 1**

Housing conditions. The calves were housed for about a week in each pen up to pen 6. Groups from pens 1 and 2 were combined in pen 3, groups from pens 4 and 5 were combined in pen 6. WM, whole milk; MR, milk replacer; B, teat bucket; A, automatic feeder.

Pen	Pen size (m <sup>2</sup> )	Number of animals/pen	Milk provision
Single pen	1.6	1	2 × 3 l/day WM, B
1 and 2	13.3	6–7	8 l/day MR, A
3	19.9	12–14	8 l/day MR, A
4	28.7	12–14	8 l/day MR, A
5	28.7	12–14	8 l/day MR, A
6	45.4	26	8–6 l/day MR, A <sup>a</sup>
Weaning pen	55.1	26	6–0 l/day MR, A <sup>b</sup>
Young stock barn	112.4	26	–

<sup>a</sup> The milk ration was reduced from 8 l to 6 l per day over the course of 9 days.

<sup>b</sup> When the youngest calf of each group was 43 days old, the calves were weaned over the course of 27 days.

day, the experimenters equipped the calves with heart rate recording devices and inter-beat intervals were measured for about 4 h (see Section 2.7). A three-phase arena test (Lürzel et al., in press; Boivin et al., 2000) was conducted on the following two mornings; however, these behavioural data will be presented in a separate publication. ADs were measured again after disbudding and after weaning. Following the AD tests after weaning, chest girth was determined as an approximation of body weight (Ulutas et al., 2002; Unalan, 2009) and heart rate was measured a second time over a period of about 4 h. Due to a limited number of heart rate monitors, it was not possible to perform these measurements on all animals in one day. Half the group was tested on the same day after the AD tests, and the other half on the following day. On each of those two days, a faecal sample was taken while fitting the heart rate recording devices in order to determine faecal cortisol metabolites (see Section 2.8); a second faecal sample of all animals in each group was taken on the third day.

### 2.3. Treatment

Gentle interactions took place between 07:00 h and 11:45 h in the single pens as well as pens 1, 2 and 3, depending on the age of the animals. They consisted in stroking of the ventral neck (Schmied et al., 2008a) and talking in a soft, soothing way. Each calf experienced gentle interactions with two experimenters (both female, dark blond hair, green overall; experimenter A: 1.80 m, experimenter B: 1.65 m). The experimenter entered the pen and remained standing close to the entrance for 10 s. Then she started talking to the calf in a gentle voice and approached it. Depending on the behaviour of the calf, she crouched or stood close to the calf, talking to it and stroking it on the ventral neck (about 60 strokes/min in each direction) for 3 min. The experimenter then slowly stood up and left the pen or, in group housing, continued to stroke another calf. Every time a calf avoided the experimenter, she waited for 10 s and then approached again. Control calves were visually separated from the treatment calves during gentle interactions. They were, however, able to watch the handlers during preparations of the

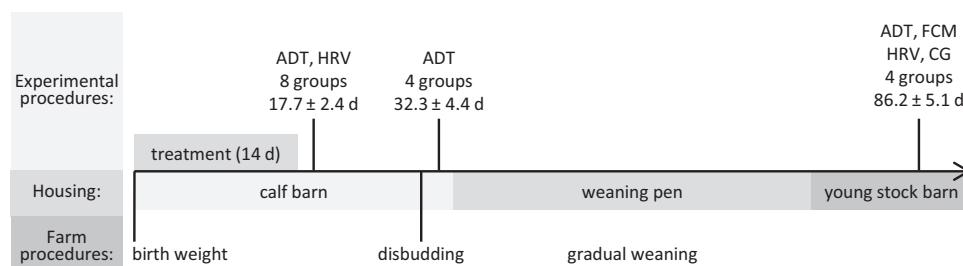
treatment and tests and thus had ample opportunities for familiarization with the experimenters without any physical contact.

### 2.4. Reaction of calves to gentle interactions

The behaviour of the stroked calves directly after entering the pen and during the treatment was scored in order to assess whether the calves perceived the treatment as positive. Scores were assigned as follows: after entering: 1 = approach, 2 = no reaction or both approach and avoidance, 3 = avoidance; during treatment: 1 = accepts stroking, 2 = accepts stroking more than half of the time (estimated), 3 = accepts stroking about half of the time, 4 = accepts stroking less than half of the time, 5 = stroking not possible (modified after Lensink et al., 2000b). Furthermore, it was noted whether the calves showed play behaviour (jumping, running, play-fighting), neck-stretching (positioning neck and head in an outstretched line, either up, down, or forward; Schmied et al., 2008b), or oral behaviours (licking and sucking on the handler's clothes and body) including butting (upward head-thrust, naturally directed against the dam's udder, in the present context against the experimenter).

### 2.5. Avoidance distance tests

Avoidance distance (AD) was measured as described by Waiblinger et al. (2002). The test was started only if the animal was standing and looking at the experimenter. The experimenter approached the animal at a speed of 1 step s<sup>-1</sup> while extending one arm in front at an angle of approximately 45°, with the back of the hand forwards. The distance between the calf's muzzle and the experimenter's hand was estimated (steps of 10 cm) at the moment when the calf avoided the experimenter by taking a step or withdrawing its head. If the calf did not avoid the experimenter, she touched the calf's nose with the back of her hand and an avoidance distance of 0 cm was assigned. During tests, a voice tracer played a recording of one sound s<sup>-1</sup> to allow for an accurate measurement of time.



**Fig. 1.** Time schedule of the experiment. ADT, avoidance distance test; HRV, measurement of heart rate to calculate heart rate variability; FCM, faecal sampling for determination of faecal glucocorticoid metabolites; CG, chest girth measure.

Experimenter A tested all calves after the treatment and after disbudding. Due to unfortunate circumstances, experimenter A was not able to test all four groups after weaning, which resulted in groups C1 and G2 being tested by experimenter A and groups G1 and C2 being tested by experimenter B. Furthermore, group C2 was still housed in a weaning pen instead of the young stock pen on the day of this test due to operational reasons. The vast majority of calves were tested between 09:00 h and 11:00 h. If it was not possible to test all animals in the morning, for instance because an animal was lying and did not stand up, testing was repeated at several points in time on the same day before attaching the heart rate recording equipment to the animals in the afternoon. The experimenter sought to test each calf twice on each testing day in order to make the data more robust.

## 2.6. Average daily gain in body weight

According to the usual practice on the farm, calves were weighed after birth. In the context of equipping the calves with the heart rate recording devices on the testing days after weaning, their approximate body weights were determined from chest girth measures. Thus, average daily gain in body weight (ADG) was calculated for the period from birth until after weaning ( $86.2 \pm 5.1$  days of age).

## 2.7. Heart rate measurements

Heart rate was recorded in the home pen using commercial non-invasive heart rate monitors (Polar Electro Oy, Kempele, Finland) in order to determine HRV. Recordings were made between 16:00 h and 21:00 h on the testing day after the treatment, and between 15:30 h and 20:00 h on the testing day after weaning. To control for effects of behavioural activity (Hagen et al., 2005), HRV was analysed only in calves that were lying as determined by video recording. The heart-rate measuring equipment was fitted to the animals according to Wagner et al. (2013), except for not shaving the skin of the animals. HRV analysis was started 1 h after the experimenter had left the pen.

Data were error-corrected and processed according to Hagen et al. (2005) using the Polar Precision Performance Software, version 4.03.050 (Polar Electro Oy, Finland) and the Kubios software, version 2.1 (Biosignal Analysis and Medical Imaging Group, Department of Applied Physics, University of Eastern Finland, Kuopio, Finland; Tarvainen et al., 2014). To account for respiratory rate (von Borell et al., 2007), frequency bands were set at 0.0033–0.04 Hz (very low frequency), 0.04–0.5 Hz (low frequency) and 0.5–0.83 Hz (high frequency). The following parameters were obtained and further analysed: mean heart rate; time domain: standard deviation of the inter-beat interval (SDNN), square root of the mean squared differences of successive inter-beat intervals (RMSSD); frequency domain (by fast Fourier transform): normalized powers for high (HFnorm) and low frequency (LFnorm), LF/HF power ratio; nonlinear domain: recurrence, determinism, Shannon entropy.

## 2.8. Faecal samples

Faecal samples were collected when the calves were equipped with the heart rate measurement devices as well as 1–2 days later at the same time of day. In total, two samples per calf were taken, each on a different day. They were collected directly from the rectum using a glove that had been wetted with water but without use of lubrication gel in order to avoid a confounding influence on faecal cortisol metabolites. After collection, the samples were put on ice and, after all animals were prepared for heart rate measurements, stored at  $-20^{\circ}\text{C}$  until analysis. Concentrations of 11,17-dioxoandrostanones, a group of cortisol metabolites, were analysed following methanolic extraction (Palme et al., 2013)

with a validated 11-oxoetiocholanolone enzyme immunoassay as described previously (Palme and Möstl, 1997; Palme et al., 1999). Intra- and inter-assay coefficients of variation were 9.5% and 11.1%, respectively, and the sensitivity of the assay was 1.7 ng/g faeces.

## 2.9. Data preparation and statistical analysis

Only data obtained from females entered the analysis, as there were only 13 males in the final sample and this number was too low to include a potential influence of sex in the analysis. We excluded data from obviously sick animals; furthermore, data were not always obtained for all females due to technical reasons. The final sample size is given in the results section, either in the text or in the figure captions. Behavioural data, ADG and concentrations of faecal cortisol metabolites were analysed and graphically presented using PASW Statistics 20 software (SPSS Inc., Chicago, IL). HRV parameters were analysed using the “lme” function from the package “nlme” (Pinheiro et al., 2013) in the statistics environment R version 3.0.3 (R Core Team, 2014). Differences, main effects, and interactions with  $p \leq 0.05$  are referred to as significant, and with  $0.05 < p \leq 0.1$  as a trend. All values presented in tables or graphs are medians and quartiles of untransformed data. When data are depicted as box plots, the bold line corresponds to the median, the lower and upper line of the box to the first and third quartile, and the whiskers to the lowest and highest values that is still within a range of 1.5 \* interquartile range. Outliers (all values between 1.5 \* interquartile range and 3 \* interquartile range) are marked with a circle, extreme values (outside of a range of 3 \* interquartile range) with an asterisk.

The two measures of AD obtained on each testing day were averaged before analysis. If AD could be measured only once, this single value was included in the analysis. AD data obtained on the testing days after the treatment and after disbudding were analysed by repeated measures ANOVA, with testing day as the repeated measure and treatment as fixed factor. Some calves had required veterinary treatments; the number of injections each calf had received before the first AD test (after the treatment) and the age at the first AD test were included as covariates. Four animals were excluded from these analyses because they had received additional injections later on, which could not be included in the calculated models. Age was removed again from the model because there was no significant effect on ADs.

Due to lack of standardization caused by different experimenters and housing conditions, AD after weaning was analysed in a separate, univariate ANOVA, with treatment and experimenter as fixed factors. The number of injections up to the third AD test and age at the third AD test were originally included as covariates in the univariate ANOVA on the AD data obtained after weaning but removed later on because they had no significant effect. For both the repeated measures and the univariate ANOVA, Levene's test was used to test for homogeneity of variances and the Shapiro–Wilk test to test for normal distribution of residuals. All AD data were square-root-transformed in order to fulfil the assumptions of ANOVA. Even after transformations, residuals of AD data obtained after disbudding were not normally distributed; main effects of testing day and treatment were, however, validated using non-parametric statistics.

ADG was analysed with a univariate ANOVA. As about half of the calves that made up each group of 26 calves had access to 8 l of milk replacer one week longer than the rest, milk provision (early reduction/late reduction) was included as a fixed factor in addition to the treatment. The age on the day of the chest girth measure and the number of injections received until then were originally included in the model but age was removed again as it did not have a significant influence. Again, assumptions of homogeneity of



variances and normal distribution of residuals were tested with the Shapiro–Wilk test and Levene's test.

As no model could be fitted to the data on faecal cortisol metabolites due to violation of the model assumptions even after transformation, groups were compared with the non-parametric Mann–Whitney *U* test.

HRV data were analysed separately for the two testing days after the treatment and after weaning, as data for both days were only available for 21 animals due to a high amount of errors in the data and resulting data loss. Treatment was included as fixed factor in the GLMM, number of injections received and body weight as covariates; as each of the covariates had a significant effect on at least one HRV parameter, they were not removed from the models. Body weight had not been directly measured at the first recording of HRV (after the treatment) and an approximation was calculated using the body weight on the day of birth and ADG (birth weight + ADG \* days of age). As random effects, we included 'animal identity' and 'group'. Model assumptions were verified by graphical inspection. A violation of the assumptions was corrected using data transformation: SDNN, LF/HF and RMSSD after weaning were log-transformed; recurrence after weaning was square-root transformed; mean heart rate after weaning, HFnorm and determinism were rank-transformed; Shannon entropy after weaning was transformed using the equation  $(\text{Shannon entropy} + 0.5)^{-3}$  (Sachs, 1997, p. 632). The results of determinism after weaning should be treated with caution, as diagnostic plots showed non-homogeneous variances regardless of the transformation. The data on several variables contained outliers producing residuals >3 SD. For these variables, models were calculated with and without the outliers to determine their influence. Only in one case, the effect of the covariate weight was affected by outliers; no main effects were affected and results of the models including the outliers are presented.

### 3. Results

#### 3.1. Behaviour during gentle interactions

In 32.1% of the 644 occasions of gentle interactions, the calves approached the experimenter, whereas in 11.5%, the first reaction towards the experimenter was avoidance. The calves let themselves be stroked the whole time in 80.1% of the occasions; there was only one calf that once accepted stroking for less than half of the time, and this animal showed very high levels of play behaviour on that occasion. Play behaviour occurred in 9.3%, oral behaviour in 44.3%, and neck stretching in 37.4% of all occasions.

#### 3.2. Avoidance distances

Regarding the AD tests after the treatment and after disbudding (Fig. 2), there were significant main effects of treatment and testing day (repeated measures ANOVA: treatment  $F_{1,72} = 5.8, p = 0.019$ ; testing day  $F_{1,72} = 96.2, p < 0.001$ ) as well as an interaction of treatment and testing day ( $F_{1,72} = 10.4, p = 0.002$ ). Stroked calves had a lower AD than controls after the treatment. After disbudding, AD was higher in both groups, and stroked calves did not differ anymore from controls. Calves that had received more injections had significantly higher ADs ( $F_{1,72} = 5.1, p = 0.026$ ).

After weaning, there was a significant main effect of experimenter (Fig. 3; ANOVA:  $F_{1,74} = 14.9, p < 0.001$ ), with lower ADs recorded by experimenter B in both stroked and control calves. There were also trends towards a main effect of treatment ( $F_{1,74} = 3.4, p = 0.07$ ) and towards an interaction of treatment and experimenter ( $F_{1,74} = 2.8, p = 0.1$ ), with lower values in stroked calves compared with controls when tested by experimenter A.

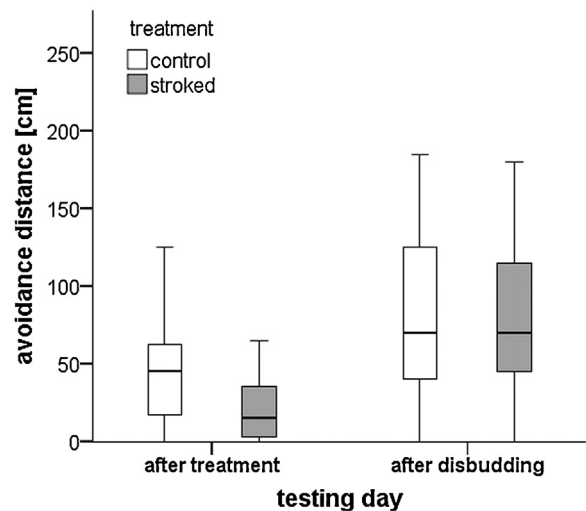


Fig. 2. Mean avoidance distance of stroked calves and controls after the treatment and after disbudding. Statistics: repeated measures ANOVA: main effect of treatment,  $p = 0.019$ ; main effect of testing day,  $p < 0.001$ , treatment \* testing day,  $p = 0.002$ ;  $n_{\text{control}} = 35, n_{\text{stroked}} = 40$ .

#### 3.3. Average daily gain in body weight

Control calves had a mean birth weight of  $39.1 \pm 5.2$  kg and a mean weight of  $122.4 \pm 14.7$  kg after weaning. In stroked calves, these values were  $39.7 \pm 4.1$  kg and  $129.9 \pm 11.5$  kg, respectively. There were significant main effects of the treatment (ANOVA:  $F_{1,67} = 4.4, p = 0.039$ ) and milk provision ( $F_{1,67} = 4.0, p = 0.048$ ) on ADG (Fig. 4) as well as an interaction of treatment and milk provision ( $F_{1,67} = 4.0, p = 0.048$ ). Calves that had access to 8 l of milk replacer one week longer had a higher ADG than calves whose milk intake was reduced earlier. Stroked calves had a higher ADG than controls, which was especially apparent in the groups that had longer access to 8 l of milk replacer. In these groups, the difference in ADG between stroked calves and controls was 6.6% (means: 1.06 kg/day vs. 0.99 kg/day). Calves that had received a higher number of injections had a significantly lower ADG ( $F_{1,67} = 9.2, p = 0.003$ ).

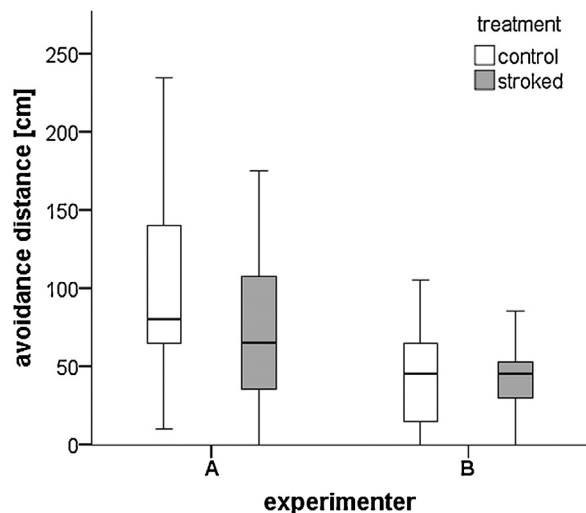
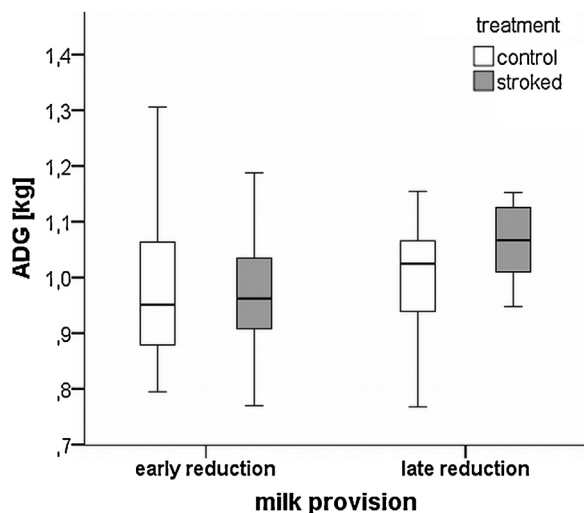


Fig. 3. Mean avoidance distance of stroked calves and controls after weaning. One of the four groups (G1; controls tested by experimenter B) was housed in a different building from the other calves on the testing day after weaning. ANOVA: main effect of experimenter,  $p < 0.001$ , main effect of treatment,  $p = 0.07$ , treatment \* experimenter,  $p = 0.1$ ;  $n_{\text{control}} = 36, n_{\text{stroked}} = 42$ .



**Fig. 4.** Average daily gain in body weight (ADG) of stroked calves and controls. Calves were weighed after birth, and their chest girth was determined after weaning ( $86.2 \pm 5.1$  days of age) as an approximation of their body weight. Early reduction/late reduction: Milk provision of every second group of 12–14 calves was reduced one week earlier from 8 l/day to 6 l/day due to the housing management. Statistics:  $n_{\text{control}} = 33$ ,  $n_{\text{stroked}} = 39$ ; ANOVA: main effect of treatment,  $p = 0.039$ ; main effect of milk provision,  $p = 0.048$ ; treatment \* milk provision,  $p = 0.048$ .

### 3.4. Heart rate variability

There was no significant main effect of the treatment on heart rate or any HRV parameters (Table 2), neither after the treatment nor after weaning. There was a trend towards a higher recurrence rate in stroked calves than in controls after weaning (GLMM:  $F_{1,2} = 12.46$ ,  $p = 0.07$ ). As effects of covariates are outside the scope of our research questions, they will not be reported.

### 3.5. Faecal cortisol metabolites

Concentrations of faecal cortisol metabolites after weaning ranged from 1.8 to 103.7 ng/g in controls and from 14.1 to 144.9 ng/g in stroked calves. There was no significant difference between groups ( $n_{\text{control}} = 24$ ,  $n_{\text{stroked}} = 35$ ; medians: control

35.7 ng/g, stroked 30.5 ng/g; Mann–Whitney  $U$  test:  $U = 391$ ,  $p = 0.66$ ).

## 4. Discussion

In this study, we tested the short-term (after the treatment) and medium-term (after disbudding, after weaning) effects of gentle interactions with group-housed dairy calves on their AD, productivity, and physiological parameters. Gentle interactions significantly decreased AD and increased ADG; they had no significant effect on HRV parameters and faecal cortisol metabolite concentrations.

### 4.1. Behaviour during gentle interactions

As in our previous study (Lürzel et al., *in press*), the calves apparently perceived the interactions with the experimenters as positive, which can be concluded from high levels of neck stretching and play behaviour. Neck stretching is shown during social licking in adult cows and interpreted as a sign of relaxation (Reinhardt et al., 1986; Schmied et al., 2010). Play behaviour did not occur as often as neck stretching; however, considering that play is known to occur irregularly and not very frequently (Jensen and Kyhn, 2000; Krachun et al., 2010), it is still quite remarkable that the calves played during the treatment in 9% of all occasions. Play behaviour is considered to be not only an indicator of positive emotions (Boissy et al., 2007) but also a means to induce them (Held and Špinka, 2011). We can therefore conclude that the gentle interactions with the experimenters were a positive experience for the calves, as already shown for dairy heifers (Bertenshaw and Rowlinson, 2008) and lambs (Tallet et al., 2005).

### 4.2. Avoidance distances

A few days after the treatment, calves that had been stroked displayed lower ADs than controls, indicating lower fear of humans as a short-term effect of the gentle interactions. This corroborates the findings of previous studies in individually housed veal calves (Lensink et al., 2000b, 2001). As expected, ADs increased after the painful experience of disbudding. The calves may have associated this procedure with the humans performing it and thus may have been more fearful of humans afterwards. An alternative explanation might be that they have a stronger tendency to avoid physical

**Table 2**  
Parameters of heart rate variability in stroked calves and controls after treatment and after weaning. After treatment  $n_{\text{control}} = 18$ ,  $n_{\text{stroked}} = 22$ ; after weaning  $n_{\text{control}} = 21$ ,  $n_{\text{stroked}} = 33$ .

Parameter	Age	After treatment			After weaning			
		Statistic Treatment	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile
Mean heart rate (beats min <sup>-1</sup> )	Control		83.8	95.7	111.9	89.9	109.3	119.0
	Stroked		74.8	95.3	102.8	103.9	111.2	117.8
SDNN (ms)	Control		26.5	33.1	48.3	16.7	22.0	39.1
	Stroked		31.4	40.5	49.2	18.6	25.1	33.0
RMSSD (ms)	Control		13.0	27.1	47.0	5.1	9.4	28.9
	Stroked		24.0	32.3	42.3	6.2	9.1	17.6
HFnorm	Control		4.0	7.8	13.6	2.2	3.6	9.8
	Stroked		3.8	7.9	13.9	2.4	5.1	8.8
LFnorm	Control		54.3	70.9	78.6	55.8	67.2	78.1
	Stroked		56.5	69.3	80.1	44.4	62.8	75.5
LF/HF	Control		5.0	9.3	16.5	7.8	17.4	31.7
	Stroked		4.9	8.2	17.7	7.3	9.8	25.3
Determinism	Control		99.0	99.6	99.8	99.0	99.7	99.8
	Stroked		97.6	99.4	99.8	99.5	99.7	99.9
Recurrence	Control		46.3	54.1	61.3	43.3	48.2	50.0
	Stroked		36.2	50.0	60.7	47.7	51.5	55.7
Shannon entropy	Control		3.6	4.1	4.4	3.6	3.9	4.0
	Stroked		3.4	3.9	4.2	3.9	4.1	4.3

contact because of the pain they are still experiencing the day after. In our study, AD was not tested directly before disbudding to minimize the influence of multiple testing on the calves' relationship to humans. Age and group size are therefore potential confounders, as they had changed between the AD test after the treatment and the test after disbudding. However, [Taschke \(1995\)](#) compared the "tameness score" (a combined measure of approach and avoidance) of calves after disbudding with values measured one day before disbudding and similarly found reduced scores not only on the day after disbudding but also 5–6 days later.

After weaning, there was a trend towards lower ADs in stroked animals again, indicating that gentle interactions during early life might have a medium-term effect on calves' relationship to humans. This finding is in accordance with previous studies (individually housed calves: [Krohn et al., 2001](#); beef suckler herd: [Probst et al., 2012](#); calves housed in small groups: [Schütz et al., 2012](#)). Unfortunately, not all of the four groups of 26 animals could be tested under standardized circumstances. The two groups tested by experimenter B had lower ADs than the two groups tested by experimenter A. A further drawback was that the control group tested by experimenter B was housed in a different building with a different pen design on the day of testing than the other three groups due to operational reasons, which may have influenced their ADs. This circumstance might explain the trend towards an interaction of experimenter and treatment. Although our study provides indications that there may be longer lasting effects of gentle interactions on the relationship between group-housed calves and humans under conditions of a large commercial farm, we cannot draw a definite conclusion from the data, and further investigations into the medium- and long-term effects of positive interactions are needed.

#### 4.3. Average daily gain in body weight

Gentle interactions had a positive effect on ADG. This effect was more pronounced in the groups that had longer access to 8 l of milk replacer before the reduction to 6 l; within these groups, the mean ADG was 0.07 kg/day higher than in stroked calves (6.6%). According to a recent meta-analysis ([Soberon and Van Amburgh, 2013](#)), there is a strong positive relationship between average daily gain until weaning and later milk yield, with calves with a 0.1 kg/day higher average daily gain producing on average 155 kg more milk during their first lactation. Thus, gentle interactions between stockpeople and calves may also be beneficial from an economic point of view. Our finding is in contrast to [Lensink et al. \(2000c\)](#), who found no effect of gentle interactions provided to veal calves after feeding twice daily over the entire fattening period (21 weeks). This difference may be due to variations in experimental design, e.g. which body region was stroked ([Schmied et al., 2008a, 2008b](#)) or, as our results imply, differences in the feeding regime. One mechanism that may lead to a higher weight gain in stroked calves is that they can allocate more energy to growth because they have lower fear responses towards humans and thus, lower energetic costs ([Turner et al., 2011](#)). Immediate physiological effects of tactile stimulation, for instance a release of oxytocin or gastrointestinal hormones ([Field, 2001](#); [Holst et al., 2005](#)), might also contribute to a better weight gain and merit further investigation. The very weak effect on daily gain of calves that had shorter access to 8 l of milk per day may be explained by a shortage of resources: the amount of nutrients contained in 6 l of milk daily may be so small that no additional growth can be induced by tactile stimulation.

#### 4.4. Physiological data

Physiological data did not differ significantly between the groups on the two testing days, neither after the treatment nor after weaning. In our study, both measurements reflect the

physiological state of the calves at a point in time where no human was present (HRV: ca. 17:00–20:00 h; cortisol metabolites: ca. 12 h before sampling, that is 02:00–04:00 h) and thus, allow conclusions about potential chronic rather than acute effects on both adrenocortical activity and the autonomic nervous system. HRV and faecal cortisol metabolites may be used to measure chronic stress in cattle (HRV: [Mohr et al., 2002](#); [Hagen et al., 2005](#); cortisol metabolites: [Huzzey et al., 2012](#); [Bertulat et al., 2013](#)) but have not been used before as such in research on the relationship between cattle and humans. Although there was no effect of the calves' relationship to humans on chronic stress in the present study, we cannot exclude acute effects on their level of stress, which would be apparent only in the presence of humans. There might also be immediate anti-stress effects of gentle interactions ([Uvnäs-Moberg, 1998](#); [Schmied et al., 2008b](#)) but they have not been studied in calves so far; as the scope of the present study did not incorporate this aspect, it remains a field for further study.

## 5. Conclusion

Gentle interactions provide an effective means to decrease calves' fear of humans in the short term and possibly also in the medium term under commercial farm conditions. They can further increase the average daily gain in body weight. We did not confirm an effect on the level of chronic stress as indicated by HRV data and concentrations of faecal cortisol metabolites. The higher average daily gain in body weight in stroked calves implicates that gentle interactions can also be beneficial from an economic point of view, as a higher average daily gain was shown to translate to a higher milk yield later in life.

## Conflict of interest

All authors declare that they have no conflict of interests.

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